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RESULTS OF PARALLEL TESTS WITH THE REITER PROTEIN COMPLEMENT-FIXATION TEST, THE TREPONEMAL IMMOBILIZATION TEST, AND THE TREPONEMAL WASSERMANN REACTION ON 1,046 SERA*

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The Treponemal Immobilization Test (TPI) is now generally accepted as a highly sensitive and specific test for treponemal infection, but its complexity and the difficulties inherent in the use of a living organism as antigen have restricted its performance to specialized laboratories. These limitations have led to the re-investigation of the cultivable strains of treponemes as antigens for the serological diagnosis of syphilis. Most workers have used the Reiter treponeme as a source of antigenic material as it is avirulent and is easily grown in artificial media. Phenolized suspensions were used by Gaehtgens (1929) and despite conflicting reports of their specificity were widely used by continental workers before the second world war. Comparisons of the results given by tests using suspensions of the intact organism as antigen with the TPI showed that such suspensions had a higher specificity and sensitivity than the classical serological tests for syphilis (STS) performed with lipoidal antigens (Benazet, Brottes, Thivolet, and Sohier, 1954; Sohier, Benazet, Brottes, and Thivolet, 1954; Hardy, Bornand, and Durel, 1955; Gastinel, Vaisman, and Hardelin, 1956; Wilkinson, 1957). Suspensions of the Reiter treponeme disintegrated by ultrasonic waves have also been found to be good antigens (Fuhner and Gaehtgens, 1954; Vaisman, Hamelin, and Prudhomme, 1958).

The preparation of four chemical fractions from the Reiter treponeme was described by D'Alessandro, Oddo, Comes, and Dardanoni (1949) and D'Alessandro and Dardanoni (1953). They isolated protein and carbohydrate fractions and two lipoids, one of which reacts with the reagin antibody detected by the standard STS using lipoidal or cardiolipin antigens. The carbohydrate fraction appears to be relatively unimportant and most attention has been paid to the thermolabile protein. This is prepared by disruption of the treponemes by cryolysis or ultrasonic waves and precipitation by exposure to increasing strengths of ammonium sulphate, followed by dialysis. The final product can be used at a fairly high titre in complement-fixation tests and is not anticomplementary, in marked contrast to suspensions of the intact organism.

De Bruijn (1956) tested sera with this antigen in parallel with the TPI test, and showed that the Reiter protein complement-fixation test (RPCFT) was both highly sensitive and specific. Subsequent reports have confirmed this (Cannefax and Garson, 1957; De Bruijn and Bekker, 1957; Rein, Kelcec, D'Alessandro, and de Bruijn, 1957; Kostant and Kelcec, 1958; Miller, Boak, and Carpenter, 1958; Foster, Nicol, and Stone, 1958; Sequeira, 1959).

Despite the advantages of the Reiter treponeme as an easily available source of antigenic material, it might be expected that the virulent organism would give a more specific antigen. Vaisman and others (1958) have compared the results of tests with protein antigens made from the Reiter and the virulent Nichols strains, using the TPI as a reference test. They claimed that the protein made from the virulent organisms had a higher specificity and sensitivity than that made from the cultivable strain. Portnoy and Magnuson (1955) introduced a test using a sodium desoxycholate extract of virulent treponemes as antigen and this appears to have a high specificity and sensitivity although the yield of antigenic material is low. Mechanically disintegrated suspensions of the Nichols strain were used as

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complement-fixing antigens in the Treponemal Wassermann Reaction (TWR) by Price and Whelan (1957) and Price (1958), and the results were found to compare favourably with the TPI.

The present investigation was undertaken to compare the results given by the RPCFT and the TWR on sera from patients known to have, or have had, syphilis, and on sera presenting diagnostic problems. Because of its high specificity and sensitivity the TPI has been used as a basis for comparison of the performance of the two tests.

Material and Methods

Tests Used

Wassermann Reaction (WR) Whitechapel technique (Price, 1949; 1950 a, b).

Price's Precipitation Reaction (PPR) (Price, 1948).

Treponemal Wassermann Reaction (TWR) (Price and Whelan, 1957).

Treponemal Immobilization Test (TPI).—The original technique of Nelson and Mayer (1949) was followed, except that the medium contained twice the amounts of sodium thioglycollate and serum ultrafiltrate and the final concentration of complement in the reaction mixture was increased to 30 per cent. Tests were read after 18 hrs incubation at 35°C.

Reiter Protein Complement-Fixation Test (RPCFT).—The antigen was used at a titre of 1 in 80 in the Whitechapel Wassermann technique, the tests being incubated at 37°C. for one hr before adding sensitized cells. Veronal buffered saline with added Ca^{++} and Mg^{++} (Mayer, Eaton, and Heidelberger, 1946) was used in place of normal saline.

Sera

These were mainly sera sent to the V.D. Reference Laboratory for TPI tests and therefore form a selected group containing many "problem" sera. Where more than one specimen from a patient was tested only the results on the first sample are included. For purposes of discussion they have been divided into the following categories:

(*A*) 149 *presumed normal adults.*—These were white blood donors whose WR and PPR had been found negative on screening tests.*

(*B*) 133 *patients diagnosed as having syphilis* (21 early, 69 latent, 21 late symptomatic, 22 congenital)

(*C a*) 466 *patients whose STS had been found positive on routine testing.*—These included 199 pregnant women and 48 blood donors.

(*C b*) 125 *patients attending the Whitechapel Clinic.*—The TWR had been found positive but the WR and PPR were negative. These sera were picked out during a clinical evaluation of the TWR by Dunlop and Price (1959).

(*D*) 236 *patients.*—These either presented lesions which might be syphilitic but with STS either negative or equivocal, or they were contacts of known syphilitics or had an indefinite history of syphilis in the past.

(*E*) 86 *lepers.*

* We are indebted to Dr. J. Shone, Director, Regional Transfusion Laboratory, Leeds for supplying these sera.

Results

(A) Presumed Normal Adults (149).—The WR, PPR, and RPCFT were negative in all cases. The TWR gave three positive reactions; TPI tests were negative on these three sera but were not carried out on the remainder of the sera in this group.

(B) Syphilitics (133).

Early Syphilis.—21 cases (17 treated). The results are shown in Table I.

TABLE I

RESULTS OF PARALLEL TESTS ON SERA FROM 21 PATIENTS WITH EARLY SYPHILIS

	TPI Result	Reactive (11)*	Negative (10)
RPCFT..	Positive ..	9	3
	Negative ..	2	7
TWR ..	Positive ..	7	4
	Negative ..	4	6
WR ..	Positive ..	6	1
	Negative ..	5	9
PPR ..	Positive ..	6	2
	Negative ..	5	8

* Sera giving positive or doubtful TPI results have been classed together as reactive in Tables I–X.

In three of the four untreated patients all the tests were positive, and the TWR was found negative in the remaining case, the other tests all being positive.

Latent Syphilis.—45 cases (untreated). The results are shown in Table II.

TABLE II

RESULTS OF PARALLEL TESTS ON SERA FROM 45 PATIENTS WITH UNTREATED LATENT SYPHILIS

	TPI Result	Reactive (45)	Negative (0)
RPCFT..	Positive ..	41	.
	Negative ..	4	.
TWR ..	Positive ..	37	.
	Negative ..	8	.
WR ..	Positive ..	38	.
	Negative ..	7	.
PPR ..	Positive ..	26	.
	Negative ..	19	.

Latent Syphilis.—24 cases (treated). The results are shown in Table III.

TABLE III

RESULTS OF PARALLEL TESTS ON SERA FROM 24 PATIENTS WITH TREATED LATENT SYPHILIS

TPI Result		Reactive (21)	Negative (3)
RPCFT..	Positive ..	14	1
	Negative ..	7	2
TWR ..	Positive ..	15	3
	Negative ..	6	0
WR ..	Positive ..	14	2
	Negative ..	7	1
PPR ..	Positive ..	11	1
	Negative ..	10	2

The patient with a positive RPCFT and negative TPI had been treated 7 years previously.

Late Symptomatic Syphilis.—21 cases (9 treated). The results are shown in Table IV.

TABLE IV

RESULTS OF PARALLEL TESTS ON SERA FROM 21 PATIENTS WITH LATE SYMPTOMATIC SYPHILIS

TPI Result		Reactive (20)	Negative (1)
RPCFT..	Positive ..	18	0
	Negative ..	2	1
TWR ..	Positive ..	11	1
	Negative ..	9	0
WR ..	Positive ..	16	1
	Negative ..	4	0
PPR ..	Positive ..	12	1
	Negative ..	8	0

The one TPI-negative patient in this group had subacute bacterial endocarditis and aortic stenosis. Syphilitic aortitis was found at autopsy.

Congenital Syphilis.—22 cases. This group included patients with signs of congenital syphilis or those in whom the diagnosis had been made in the past and treatment given. The results are shown in Table V.

TABLE V

RESULTS OF PARALLEL TESTS ON SERA FROM 22 PATIENTS WITH CONGENITAL SYPHILIS

TPI Result		Reactive (20)	Negative (2)
RPCFT..	Positive ..	12	0
	Negative ..	8	2
TWR ..	Positive ..	11	0
	Negative ..	9	2
WR ..	Positive ..	12	1
	Negative ..	8	1
PPR ..	Positive ..	5	0
	Negative ..	15	2

Considering the group of syphilitic patients as a whole, Tables I–V show that, while the TPI had the highest sensitivity of the five tests used, the RPCFT approached it most closely. Out of the total of 133 sera, the TPI was positive or doubtful in 118 instances, the RPCFT positive in 98, the TWR in 89, the WR in 91, and the PPR in 64.

(C) **Problem Sera** (466).—This group included 199 pregnant women and 48 blood donors who had been found sero-positive on routine testing. The remaining 219 patients were suffering from a wide variety of conditions but all had been found to have positive STS and confirmatory tests were therefore requested. The results of the parallel tests are summarized in Table VI.

TABLE VI

RESULTS OF PARALLEL TESTS ON SERA FROM 466 PATIENTS FOUND SERO-POSITIVE ON ROUTINE TESTING

TPI Result		Reactive (239)	Negative (227)
RPCFT..	Positive ..	186	2
	Negative ..	53	225
TWR ..	Positive ..	165	74
	Negative ..	74	153
WR ..	Positive ..	178	88
	Negative ..	61	139
PPR ..	Positive ..	137	60
	Negative ..	102	167

These figures show clearly that the RPCFT agreed more closely with the TPI than any of the other tests used, particularly in the TPI-negative group in which only two sera gave discordant results in comparison with 60 to 88 sera with the other tests. One of these sera came from a pregnant woman aged 36 with corneal opacities and deafness who had been treated with penicillin; the RPCFT was the only positive test in this case. The other serum was from a woman aged 46 whose husband had had early syphilis in 1946 and now had tabes. In this case also, the RPCFT was the only test giving a positive result. It would seem that syphilis could be excluded in neither of these cases.

In connexion with a clinical evaluation of the TWR (Dunlop and Price, 1959) this test was performed on all new cases attending the Whitechapel Clinic, London. In 125 instances the TWR had been found positive while the WR and PPR were both negative and TPI tests had been requested because of this discrepancy. RPCFTs were also performed on these sera and the results are shown in Table VII (overleaf).

45 of the patients in this group were white, 57 came from the West Indies or West Africa, and the

TABLE VII

RESULTS OF PARALLEL TESTS ON SERA FROM 125 PATIENTS ATTENDING THE WHITECHAPEL CLINIC WHO HAD SHOWN ISOLATED POSITIVE TWR TESTS ON A PREVIOUS SPECIMEN OF SERUM

TPI Result		Reactive (28)	Negative (97)
RPCFT..	Positive ..	18	2
	Negative ..	10	95
TWR ..	Positive ..	23	75
	Negative ..	5	22
WR ..	Positive ..	2	0
	Negative ..	26	97
PPR ..	Positive ..	0	0
	Negative ..	28	97

majority of the remainder were Indians or Pakistanis. Five of the white patients were found to have a history of treated syphilis. The TPI was reactive in two of these, the TWR in three, and the RPCFT in one. Another white male aged 62 with a urethral stricture had a doubtful TPI result on three occasions; the TWR was positive but the other tests negative.

The case records of the patients from the West Indies (53) and West Africa (4) were examined for data regarding past infection with yaws. In eighteen cases there was a previous history of this disease, usually treated during infancy or childhood, a further 27 patients denied ever having had yaws; in the remaining twelve cases there was either no information available or previous infection with yaws had only been queried. A comparison of the results of the three treponemal tests with the patients' histories is shown in Table VIII.

TABLE VIII

COMPARISON OF THE RESULTS OF THE TREPONEMAL TESTS WITH A PREVIOUS HISTORY OF YAWS

Test	Result	Previous History of Yaws				
		Yes (18)		No (27)		No Definite Information (12)
		No.	Per cent.	No.	Per cent.	
TPI ..	Reactive	13	72	6	22	5
	Negative	5		21		7
RPCFT	Positive	10	55	6	22	2
	Negative	8		21		10
TWR..	Positive	15	83	23	85	10
	Negative	3		4		2

Although the numbers are small, it is clear that reactive TPI and RPCFT tests were found more frequently among those patients who gave a history of yaws in the past. The agreement between these two tests was close, only two patients showing a positive RPCFT in association with a negative TPI test; both were West Indians, one with and one without a past history of yaws. The positive TWR tests were equally distributed between the patients with a past history of yaws and those without, but as the group had been selected because the TWR had been found positive, no conclusions can be drawn from this.

(D) Possible Syphilitics (236).—These patients either had lesions which were thought to be possibly due to syphilis or they were the sexual partners of known syphilitics. In the majority of cases the STS had been found negative or equivocal and a TPI test was requested to clarify the position. A small proportion of the patients had an indefinite history of previous syphilis and had received some treatment in the past. The results of the parallel tests are shown in Table IX.

TABLE IX

RESULTS OF PARALLEL TESTS ON SERA FROM 236 PATIENTS WHOSE LESIONS OR HISTORY AROUSED SUSPICION OF SYPHILIS

TPI Result		Reactive (76)	Negative (160)
RPCFT..	Positive ..	55	4
	Negative ..	21	156
TWR ..	Positive ..	49	22
	Negative ..	27	138
WR ..	Positive ..	38	13
	Negative ..	38	147
PPR ..	Positive ..	29	5
	Negative ..	47	155

The results obtained in this group of sera also show that the RPCFT agreed more closely with the TPI than did the other tests. Four sera gave positive RPCFTs although the TPI was found to be negative. Three of these patients had a history of treatment for syphilis 7, 33, and 40 years previously. The fourth was a man aged 58 with a tumour of the larynx whose STS had given equivocal results.

(E) Lepers (86).—Tests were also performed on sera from 86 patients with leprosy of the lepromatous type.* The results are shown in Table X (opposite).

Leprosy, particularly the lepromatous form, is well known to be frequently associated with non-specific STS reactions. The RPCFT gave no positive reactions which were not accompanied by a positive TPI test. The high proportion of positive TWR tests in cases in which the TPI was found to be negative is noteworthy.

* We are indebted to Dr. J. A. McFadzean of the Sungei Buloh Settlement, Selangor, Malaya, for making these sera available to us.

TABLE X

RESULTS OF PARALLEL TESTS ON SERA FROM 86 PATIENTS WITH LEPROSY

TPI Result		Reactive (11)	Negative (75)
RPCFT..	Positive ..	7	0
	Negative ..	4	75
TWR ..	Positive ..	8	37
	Negative ..	3	38
WR ..	Positive ..	7	3
	Negative ..	4	72
PPR ..	Positive ..	7	9
	Negative ..	4	66

Discussion

The results of the tests on sera from patients with syphilis (summarized in Tables I–V) show that, while the RPCFT was more sensitive than the TWR, WR, or PPR, it gave negative results with 23 sera in which the TPI was still positive; seventeen of these came from treated patients.

The relative specificity of the various tests as judged by the TPI can be estimated from the results of tests on "problem" sera shown in Tables VI–X. The RPCFT gave positive reactions with 274 sera and the TPI test was found to be negative in only eight of these cases, three of whom had a history of syphilis in the past while a further two aroused suspicion of syphilis on clinical grounds. Even discounting these, the RPCFT had a specificity of 97 per cent. in terms of the TPI. Of the 639 sera in the problem group giving negative RPCFT results, 88 (14 per cent.) were found to have positive TPI tests. This suggests that the complement-fixation technique used in this study may have been undersensitive and in this connexion it should be noted that other workers have used more sensitive cold-fixation techniques.

The correlation between the TWR and the TPI was found to be very low in the problem sera; 453 of these gave positive TWR results, but the TPI was positive in only 245 (54 per cent.) of these. In contrast, the TPI was found to be positive in 109 sera (24 per cent.) out of 460 in this group in which the TWR was negative. If the TPI is taken as the basis of comparison, the results obtained in this group of sera do not suggest that the TWR can be safely used as an alternative to it.

The high specificity of tests using the protein fraction of the Reiter organism as antigen found in this and other studies supports the view of D'Alessandro and Dardanoni (1953) that a group-specific antigen might be shared in common by the virulent and cultivable strains of *T. pallidum*. Dardanoni and Censuales (1957) and Cannefax and Garson (1959) have provided experimental support for this contention; the former workers showed that a protein fraction prepared from virulent *T. pallidum* (Nichols strain) can absorb antibodies from syphilitic sera reacting not only with itself but also with the protein fraction prepared from the Reiter treponeme. The latter fraction, however, can only absorb homologous antibody. They conclude that, while the virulent and cultivable strains share a common antigen, the virulent strain also possesses a specific component which is lacking in the Reiter organism. Suspensions of intact organisms can only absorb their homologous antibody from syphilitic and anti-Reiter immune sera. This suggests that the specific antigen may be located near the surface of the treponeme while the common group antigen is more deeply situated and is only set free by the methods of disintegration used in the preparation of the protein antigens. De Bruijn (1958) has shown that the protein antigen used in the RPCFT still contains a portion of the ubiquitous lipoid antigen which reacts with reagin antibody. As this lipoid is only demonstrable at low dilutions of the antigen (neat and 1 in 2·5) while in the RPCFT the antigen is used at a titre of 1 in 80, it does not appear likely that the contaminating lipoid would affect the specificity of the test provided that the antigen is used at its proper titre.

Summary

(1) Parallel tests have been carried out with the Treponemal Immobilization Test, the Reiter Protein Complement-Fixation Test, the Treponemal Wassermann Reaction, a standard Wassermann Reaction, and Price's Precipitation Reaction, on sera from 133 patients with syphilis and on 913 problem sera.

(2) In tests on syphilitic sera, the RPCFT was found to be more sensitive than the TWR and the STS used. In tests on sera which presented diagnostic problems, the RPCFT gave results which were in much closer agreement with the TPI than were those of the TWR.

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